



(11) **EP 2 947 153 A3**

(12) **EUROPEAN PATENT APPLICATION**

(88) Date of publication A3:  
**02.03.2016 Bulletin 2016/09**

(51) Int Cl.:  
**C12P 19/04<sup>(2006.01)</sup> C12R 1/01<sup>(2006.01)</sup>**

(43) Date of publication A2:  
**25.11.2015 Bulletin 2015/48**

(21) Application number: **15162029.1**

(22) Date of filing: **31.03.2015**

(84) Designated Contracting States:  
**AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**  
Designated Extension States:  
**BA ME**  
Designated Validation States:  
**MA**

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(30) Priority: **01.04.2014 IN DE09392014**

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(54) **A PROCESS FOR THE PREPARATION OF POLYMERIC BIOSURFACTANTS**

(57) The present invention relates to polymeric biosurfactants isolated from two bacterial strains of *Microbacterium* sp. strain BS-2 [MTCC 5822] and *Brevibacillus* sp. strain BS-207 [MTCC 5823]. The present invention relates to an acidic exopolysaccharide (EPS), termed Microsan, with chemical composition of glucose, mannose and glucuronic acid ( $\beta$ -D-glucuronyl-(1-2)-D-manno-

syl-(1-4)-D-glucose) produced by *Microbacterium* sp. strain BS-2 and a neutral EPS, termed Brevisan, of galactomannan with galactose and mannose residues in the ratio of 1:1 produced by *Brevibacillus* sp. strain BS-207. Both these polysaccharides exhibited surface-active and potential antibacterial, antioxidant, anti-inflammatory, and immunomodulatory activities.

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The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (IPC) C12P C12R
Place of search The Hague		Date of completion of the search 18 January 2016	Examiner van de Kamp, Mart
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 18 January 2016	Examiner van de Kamp, Mart
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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A,D	KUMAR A S ET AL: "Bacterial exopolysaccharides - a perception", JOURNAL OF BASIC MICROBIOLOGY, vol. 47, no. 2, 1 April 2007 (2007-04-01), pages 103-117, XP002560843, ISSN: 0958-6946, DOI: 10.1002/JOBM.200610203 * the whole document * -----	1-16	
			TECHNICAL FIELDS SEARCHED (IPC)
The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 18 January 2016	Examiner van de Kamp, Mart
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**CLAIMS INCURRING FEES**

The present European patent application comprised at the time of filing claims for which payment was due.

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Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due and for those claims for which claims fees have been paid, namely claim(s):

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No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for those claims for which no payment was due.

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**LACK OF UNITY OF INVENTION**

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

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see sheet B

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All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

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As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

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Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

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None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

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The present supplementary European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims (Rule 164 (1) EPC).

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**LACK OF UNITY OF INVENTION  
SHEET B**

Application Number  
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The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 2, 8, 9, 13, 15(completely); 1, 4-7, 10, 11(partially)

Polymeric biosurfactant isolated from bacterial strains and characterized in that they are exopolysaccharide (EPS) in nature, further characterized in having glucose, mannose and glucuronic acid residues in beta-(1-4), (1-2) configuration, an average molecular weight (Mw) of 36663 KDa, intrinsic viscosity of 1.0 dL/g and showing random coil confirmation. Said polymeric biosurfactant which reduces and stabilizes gold nanoparticles at 0.01% concentration to obtain polymeric biosurfactant-capped gold nanoparticles. A method for producing said polymeric biosurfactant under aerobic conditions in an aqueous nutrient medium comprising the steps of: isolating bacterial strain *Microbacterium* sp. strain BS-2 (MTCC 5822) from petroleum contaminated soil using N-hexadecane (1%, v/v); b. subjecting the isolated strains as obtained in step (a) to grow in the fermentation media at temperature in the range of 35 to 40°C for period in the range of 45 to 50 hr; c. centrifuging the culture as obtained in step (b) at temperature in the range of 3 to 5°C to obtain polymeric biosurfactant. An isolated *Microbacterium* sp. strain BS-2 having accession number MTCC 5822. An isolated *Microbacterium* sp. strain BS-2 having accession number MTCC 5822, for use in generating said polymeric biosurfactant.

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2. claims: 3, 12, 14, 16(completely); 1, 4-7, 10, 11(partially)

Polymeric biosurfactant isolated from bacterial strains and characterized in that they are exopolysaccharide (EPS) in nature, further characterized in having galactose and mannose residues in beta-(1-4) configuration, an average molecular weight (Mw) of 42260 KDa, intrinsic viscosity of 1.0 dL/g and showing triple helical confirmation. A method for producing said polymeric biosurfactant under aerobic conditions in an aqueous nutrient medium comprising the steps of: isolating bacterial strain *Brevibacillus* sp. strain BS-207 (MTCC 5823) from petroleum contaminated soil using N-hexadecane (1%, v/v); b. subjecting the isolated strains as obtained in step (a) to grow in the fermentation media at temperature in the range of 35 to 40°C for period in the range of 45 to 50 hr; c. centrifuging the culture as obtained in step (b) at temperature in the range of 3 to 5°C to obtain polymeric biosurfactant. An isolated *Brevibacillus* sp. strain BS-207 having accession number MTCC 5823. An isolated *Brevibacillus* sp. strain BS-207 having accession number MTCC 5823, for use in generating said polymeric biosurfactant.

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ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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